Neuregulin and dopamine modulation of hippocampal gamma oscillations is dependent on dopamine D4 receptors

Richard H. Andersson[a], April Johnston[a], Paul A. Herman[b], Ursula H. Winzer-Serhan[c], Irina Karavanova[b], Detlef Vullhorst[b], André Fisahn[a], and Andres Buonanno[b,1]

[a]Neuronal Oscillations Laboratory, KI-Alzheimer Disease Research Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institute, 14186 Stockholm, Sweden; [b]Section on Molecular Neurobiology, Eunice Shriver Kennedy National Institute of Child Health and Human Development, Bethesda, MD 20892; and [c]Department of Neuroscience and Experimental Therapeutics, Health Sciences Center, College of Medicine, Texas A&M University System, Bryan, TX 77807

Edited* by Gerald D. Fischbach, The Simons Foundation, New York, NY, and approved June 26, 2012 (received for review January 19, 2012)

The neuregulin/ErbB signaling network is genetically associated with schizophrenia and modulates hippocampal γ oscillations—a type of neuronal network activity important for higher brain processes and altered in psychiatric disorders. Because neuregulin-1 (NRG-1) dramatically increases extracellular dopamine levels in the hippocampus, we investigated the relationship between NRG/ErbB and dopamine signaling in hippocampal γ oscillations. Using agonists for different D1- and D2-type dopamine receptors, we found that the D4 receptor (D4R) agonist PD168077, but not D1/D5 and D2/D3 agonists, increases γ oscillation power, and its effect is blocked by the highly specific D4R antagonist L-745,870. Using double in situ hybridization and immunofluorescence histochemistry, we show that hippocampal D4R mRNA and protein are more highly expressed in GAD67-positive GABAergic interneurons, many of which express the NRG-1 receptor ErbB4. Importantly, D4 and ErbB4 receptors are coexpressed in parvalbumin-positive basket cells that are critical for γ oscillations. Last, we report that D4R activation is essential for the effects of NRG-1 on network activity because L-745,870 and the atypical antipsychotic clozapine dramatically reduce the NRG-1–induced increase in γ oscillation power. This unique link between D4R and ErbB4 signaling on γ oscillation power, and their coexpression in parvalbumin-expressing interneurons, suggests a cellular mechanism that may be compromised in different psychiatric disorders affecting cognitive control. These findings are important given the association of a *DRD4* polymorphism with alterations in attention, working memory, and γ oscillations, and suggest potential benefits of D4R modulators for targeting cognitive deficits.

fast-spiking interneuron | attention-deficit/hyperactivity disorder | cognitive enhancers | excitatory/inhibitory balance

Gamma oscillations (30–80 Hz) are rhythmic local field potentials that synchronize local circuit activity and play an important role in higher brain processes, such as learning, memory, and cognition. Impairments in general synchronized network activity, in particular γ oscillations, are core features of several neurological and psychiatric disorders, such as schizophrenia, in which perception, cognition, and working memory are affected (1, 2). γ oscillation power is impaired during cognitive tasks in first-episode schizophrenia independent of medication, indicating that these alterations are independent of disease history (3).

Pharmacological studies in anesthetized rats and genetic studies in mutant mice emphasize the importance of recurrent excitatory and inhibitory interactions for the generation of γ oscillations in hippocampal networks (4). The development of methodologies to study neural network activity in acute hippocampal slices in vitro, whereby γ oscillations are induced by the activation of metabotropic glutamate receptors (5), muscarinic acetylcholine receptors (6), or kainate (KA) receptors (7), has been instrumental to identify cellular and molecular mechanisms underlying the generation and regulation of γ oscillations (8, 9). In particular, bistratified cells and fast-spiking parvalbumin-positive (PV+) GABAergic interneurons have been implicated in the generation of γ oscillations in area CA3 of the hippocampus and in the cortex (10). Targeted ablation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors selectively in PV+ interneurons impairs hippocampal network synchrony and results in deficits in spatial and working memory (11, 12). Consistent with these findings, optogenetic activation of PV+ interneurons is sufficient to modulate γ oscillations, cortical circuit synchrony, and behaviors (13, 14).

Although it has long been appreciated that dopamine (DA) influences processes underlying attention, cognitive salience, and working memory (15), and that DA levels are altered in schizophrenia, relatively little is known about the effects of DA on γ oscillation activity in the hippocampus and frontal cortex. The rodent frontal cortex and hippocampus receive sparse dopaminergic innervation from the ventral tegmental area that regulates synaptic transmission and plasticity (16). We recently found that signaling via neuregulin-1 (NRG-1) and its cognate transmembrane receptor ErbB4, both genetically associated with increased risk for schizophrenia (17), dramatically increases extracellular DA levels and regulates hippocampal synaptic plasticity via DA D4 receptor (D4R) activation (18). We also reported that NRG-1/ErbB4 signaling greatly augments the power of KA-induced γ oscillations in acute hippocampal slices (19). Ablation of ErbB4, which is expressed in rodent and primate hippocampal (19, 20) and cortical (21) GABAergic interneurons (including PV+ interneurons), blocks the effects of NRG-1 and reduces γ oscillation power by more than 50% (19). Taken together, these findings suggested a potential interaction between the NRG-1/ErbB4 and DA signaling pathways in the regulation of γ oscillations in the hippocampus. Therefore, in the present study we have investigated the role of DA receptor subtypes (D1–D5), as well as their interaction with the NRG-1/ErbB4 signaling pathway, in regulating KA-induced γ oscillations.

Results

D1- and D2-Type Receptor Expression in the Hippocampus. Using sensitive semiquantitative reverse transcription PCR (qRT-PCR) to compare the relative mRNA levels of D1–D5 receptors in the frontal cortex and the hippocampus, we found that DA receptor

Author contributions: R.H.A., A.J., U.H.W.-S., I.K., A.F., and A.B. designed research; R.H.A., A.J., P.A.H., U.H.W.-S., I.K., D.V., A.F., and A.B. performed research; R.H.A., A.J., P.A.H., U.H.W.-S., I.K., D.V., A.F., and A.B. analyzed data; and R.H.A., D.V., A.F., and A.B. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: buonanno@mail.nih.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1201011109/-/DCSupplemental.

subtypes are vastly different (Fig. S1). In the hippocampus, D2-type DA receptor transcript levels are 10- to 1,000-fold lower than D1-type receptor mRNAs (D1/β-actin: 2.4 ± 0.72E-03; D5/β-actin: 7.5 ± 2.0E-03). Among the D2-type DA receptors, D2 is the most abundant, (D2/β-actin: 7.0 ± 1.4E-04), followed by D4 (D4/β-actin: 4.1 ± 0.6E-05). Transcripts for D3 are extremely low (D3/β-actin: 2.0 ± 1.0E-06).

Effects of DA Receptor Subtype Activation on KA-Induced γ Oscillations. D1-type and D2-type DA receptors are positively and negatively coupled to adenylate cyclase, respectively, and frequently mediate opposing effects on distinct functions in the cortex (22, 23) and hippocampus (16, 17). To investigate the effects of DA on hippocampal network activity, γ oscillations were induced with 100 nM KA, and slices were then perfused with either 10 nM, 10 μM, or 200 μM DA. No significant increase of γ oscillation power was observed at these DA concentrations (Fig. 1 *A* and *E* and Table S1). Next, we pharmacologically dissected the possible involvement of D1- and D2-type receptor subtypes. When DA was added to slices treated with KA and the selective D1-type receptor antagonist SCH23390 (300 nM), γ oscillation power increased from 1.99E-09 ± 9.32E-10 V^2 to 2.56E-09 ± 1.08E-09 V^2 ($n = 7$, $P = 0.0313$) without effects on peak frequency (Fig. 1*B*); normalized γ power increased to 141.19% ± 15.37% (Fig. 1*E*). This result suggested that an effect of DA on γ oscillations mediated through D2-type receptors could be unmasked by blocking D1-type receptors. To further identify the specific DA receptor subtype(s) capable of modulating γ oscillations, we used selective agonists for D1/D5 and D2/D3 receptors. As shown in representative traces and power spectra (Fig. 1*C*), perfusion of hippocampal slices with the D1/D5 receptor-selective agonist SKF81297 (1 μM) affected neither frequency nor amplitude of γ oscillations (KA: 3.36E-09 ± 9.51E-10 V^2 vs. KA+SKF81297: 3.93E-09 ± 1.40E-09 V^2; $n = 14$, $P = 0.5016$). Next, slices were perfused with 7-hydroxy-DPAT (7-OH-DPAT; 100 nM), a selective D2/D3 receptor agonist. As shown in Fig. 1 *D* and *E*, 7-OH-DPAT had no significant effect on KA-induced γ oscillation power (KA: 8.26E-09 ± 2.34E-09 V^2 vs. KA+7-OH-DPAT: 1.33E-08 ± 2.35E-09 V^2; $n = 10$, $P = 0.23$).

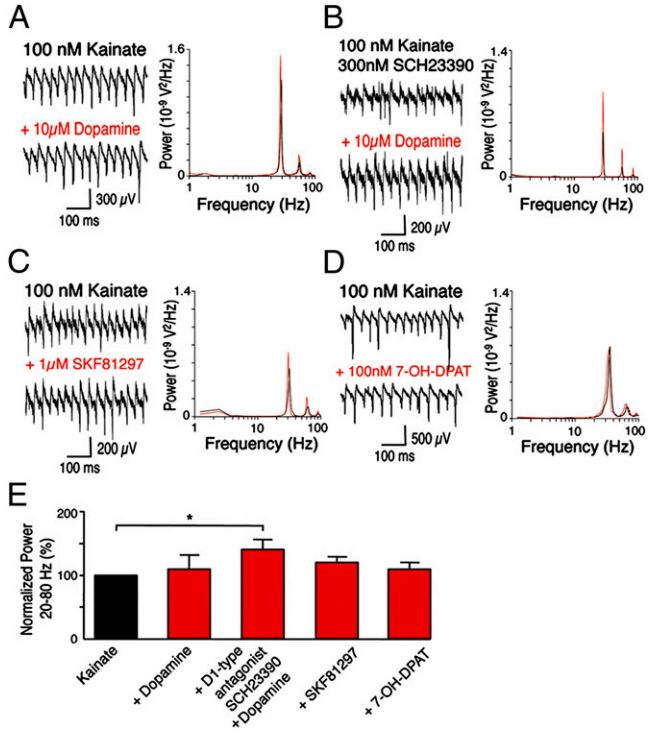

Fig. 1. Analysis of KA-induced γ oscillations in rat hippocampal slices after application of DA and selective D1- and D2-type receptor agonists/antagonists. Representative sample traces (*Left*) and power spectra (*Right*) of KA-induced γ oscillations in rat slices; the color of lines in power spectra correspond to the type of treatment indicated over each sample trace (*Right*). (*A*) DA (10 μM) was added after the induction of γ oscillations with 100 nM KA ($n = 5$). No change was observed with DA. (*B*) Effect of the D1-type selective antagonist SCH23390 (300 nM) on KA-induced γ oscillations in presence of 10 μM DA ($n = 7$). (*C*) Lack of effect of the D1/D5 receptor agonist SKF81297 (1 μM; $n = 14$). (*D*) Lack of effect of the D2/D3 receptor-selective agonist 7-OH-DPAT (100 nM; $n = 10$). (*E*) Quantification of normalized KA-induced γ oscillation power in response to the above treatments. Inclusion of the D1/D5 receptor-selective antagonist SCH23390 to the bath solution significantly increased normalized γ oscillation power by 42% ($P = 0.013$).

D4R Activation Augments KA-Induced γ Oscillations. In contrast to the D1/D5 and D2/D3 receptor agonists, the D4R selective agonist PD168077 (100 nM) significantly augmented normalized γ oscillation power to 140.65% ± 9.86% (KA: 5.11E-09 ± 1.88E-09 V^2 vs. KA+PD168077: 8.53E-09 ± 3.99E-09 V^2; $n = 13$, $P = 0.0024$; Fig. 2 *A* and *D*) without affecting oscillation peak frequency (KA: 32.07 ± 1.24 Hz vs. KA+PD168077: 30.80 ± 1.07 Hz; $n = 13$, paired t test, $P = 0.1751$). No activity pattern was either generated or changed when PD168077 was perfused onto naïve slices (ACSF only: 6.50E-11 ± 3.22E-11 V^2 vs. ACSF + PD168077: 3.89E-11 ± 1.52E-11 V^2; $n = 4$, $P = 0.125$).

Having identified the D4R as being responsible for the increase in γ power, we revisited the question of why DA alone does not produce an increase in power. Indeed, in the presence of the D1/D5 receptor agonist SKF81297 (1 μM), PD168077 (100 nM) was no longer able to increase power (SKF81297: 1.33E-09 ± 4.98E-10 V^2 vs. SKF81297+PD168077: 1.23E-09 ± 4.21E-10 V^2; $n = 6$, $P = 0.56$). This finding suggests that D1/D5 receptors oppose the ability of D4Rs to augment γ power (compare with DA application experiment, Fig. 1 *A* and *E* and Table S1). It also explains why DA increases γ power in the presence of the D1/D5 receptor blocker SCH23390 (Fig. 1*E*).

Effects of PD168077 Are Specific for D4R. To affirm that PD168077 effects on γ oscillations are mediated by D4Rs, we included the D4R-selective antagonist L-745,870 (500 nM). As shown in Fig. 2 *B* and *D*, PD168077 failed to increase γ oscillation power when slices were perfused with KA and L-745,870 (KA+L-745,870: 3.61E-09 ± 1.11E-09 V^2 vs. KA+L-745,870+PD168077: 3.53E-09 ± 1.18E-09 V^2; $n = 6$, $P = 0.4375$). In contrast, the D2/D3-receptor selective antagonist sulpiride (500 nM) did not affect the response to PD168077 (KA+sulpiride: 3.22E-08 ± 0.86E-08 V^2 vs. KA+sulpiride+PD168077: 2.28E-08 ± 0.44E-08 V^2; $n = 6$, $P = 0.04$; Fig. 2 *C* and *D*). Importantly, when used on their own, no significant effects of DA receptor antagonists on KA-induced γ oscillation power were observed for the D4R antagonist (500 nM L-745,870: KA: 1.01E-09 ± 5.77E-10 V^2 vs. KA+L-745,780: 3.61E-09 ± 1.11E-09 V^2; $n = 6$, $P = 0.16$) or antagonists selectively targeting either D1/D5 receptors (300 nM SCH23390, KA: 1.77E-09 ± 8.70E-10 V^2 vs. SCH23390: 1.99E-09 ± 9.92E-10 V^2; $n = 7$, $P = 0.08$) or D2/D3 receptors (500 nM sulpiride, KA: 2.078E-09 ± 9.392 E-10 V^2 vs. sulpiride: 2.076E-09 ± 9.397 V^2; $n = 7$, $P = 0.999$). Taken together, our results indicate that activation of D4Rs, but not D2/D3 or D1/D5 receptors, selectively increases the power of hippocampal KA-induced γ oscillations.

D4R Transcripts Are Coexpressed with GAD67-Positive Cells in the Hippocampus. The expression pattern of D4R protein in the hippocampus is controversial (see refs. 24 and 25 vs. 26). Therefore, we began by using isotopic in situ hybridization with an antisense ^{35}S-labeled D4R cRNA to investigate receptor expression in the hippocampus. Emulsion autoradiography with dark-field analysis had to be used to detect the sparse positive cells. D4R transcripts

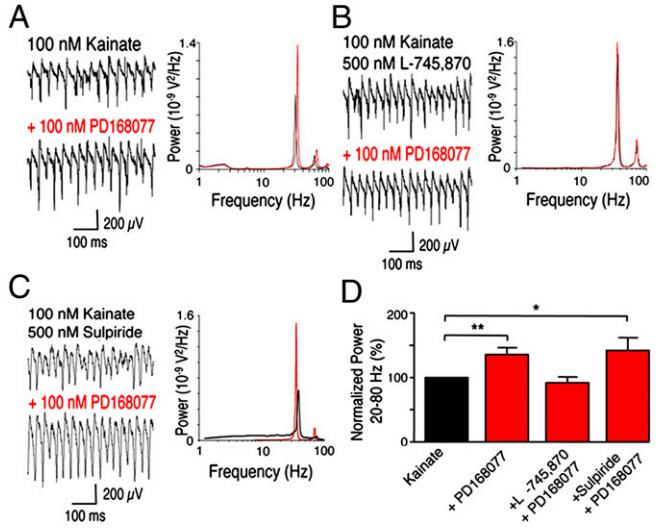

Fig. 2. Effects of PD168077 on KA-induced γ oscillations are blocked by a specific D4R, but not D2/D3 receptor, antagonists. Representative sample traces (*Left*) and power spectra (*Right*) of KA-induced γ oscillations in rat slices; line colors in power spectra correspond to the type of treatment indicated over each sample trace (*Right*). (*A*) The D4R-selective agonist PD168077 (100 nM) significantly increases the power of KA-induced γ oscillations ($n = 13$). (*B*) Prior application of the D4R antagonist L-745,870 (500 nM; $n = 6$) prevents the PD168077-induced increase in γ oscillation power. (*C*) Prior application of the D2/D3 receptor antagonist sulpiride (500 nM; $n = 6$) does not prevent the PD168077-induced increase in γ oscillation power. (*D*) Summary analysis of normalized data shown in *A*, *B*, and *C*; power of KA-induced γ oscillations set as 100%.

accumulate in a few cells in the hippocampus and subiculum (Fig. 3*A*), presumably corresponding to GABAergic neurons, whereas principal cells in *stratum pyramidale* show very low or no signal. To confirm that the positive cells are GABAergic, double in situ hybridization (DISH) was performed with the ^{35}S-labeled D4R cRNA probe and a digoxigenin-labeled probe for GAD67. D4R transcripts clustered in a subset of GAD67-positive interneurons (Fig. 3 *B–E*) in the *cornu ammonis* and the *subiculum*; however, many GABAergic neurons were negative for the receptor. Doubled-labeled GAD67/D4R-expressing cells were observed in the *strata oriens*, *pyramidale*,and *radiatum* but not in the *stratum lucidum moleculare*.

D4R Protein Colocalizes in Cells Positive for the Neuronal Marker NeuN and for GAD67. Double immunofluorescence histochemistry (IFH) was used to analyze the cellular expression of the D4R protein. Of six independent antisera tested (*SI Materials and Methods*), an affinity-purified antibody against the N-terminal 16 amino acids of the rat D4R demonstrated an overlapping distribution with D4R transcripts (24). Immunoreactivity was sparse throughout the hippocampus, as exemplified in the two regions of interest, CA1 (Fig. S2) and CA3, shown in Fig. 3. Double-labeling with NeuN revealed that D4R immunoreactivity is restricted to neurons (Fig. 3 *F–H*) and seems to be highest in the soma and excluded from the nucleus, a subcellular distribution consistent with reports of other G protein-coupled receptors (27). Double IFH using a GAD67-specific antibody showed that most D4R-positive cells correspond to GABAergic interneurons (Fig. 3 *I–K*), consistent with our DISH results (Fig. 3 *B–E*).

D4R Is Coexpressed with ErbB4 Receptor and PV. Fast-spiking PV+ interneurons play an important role in synchronizing γ oscillations (10), and their enhanced activity is sufficient to generate γ frequency network activity in vitro (13) and in vivo (14). D4R immunoreactivity was present in a subset of PV+ interneurons in CA1 (Fig. 4 *A–C*) and CA3 (Fig. 4 *D–F*). Colabeling was most frequently observed over neuronal cell bodies residing in the *stratum pyramidale* and nearby in the *strata oriens* and *radiatum*. In these strata, we found that 71% ± 1.3% of D4R-immunoreative neurons also express PV (267 of 376 D4R neurons; $n = 8$ sections from four rats; mean ± SEM) and that D4R-positive cells represent 25% ± 1.1% of all PV neurons (376 of 1,075 PV+ neurons). That these D4R-immunoreactive neurons also express PV and reside in the *stratum pyramidale* suggests they are fast-spiking GABAergic basket cells or bistratified cells as we showed in a recent study (28).

Because ErbB4 receptors modulate KA-induced γ oscillations in response to NRG-1 and are expressed in ∼50% of PV+ neurons throughout all layers of the *cornu ammonis* (20), we investigated whether D4 and ErbB4 receptors are coexpressed. As shown in Fig. 4 *G–I*, we found a subset of D4R-immunoreactive neurons that are also ErbB4 positive. On average, 54% ± 0.9% of D4R-positive neurons also express ErbB4 (161 of 296 D4R neurons; $n = 8$ from four rats; mean ± SEM).

ErbB4 and D4 Receptor Signaling Pathways Converge to Modulate γ Oscillations. On the basis of the coexpression of D4 and ErbB4 receptors in GABAergic basket cells, the effects of NRG-1 on γ

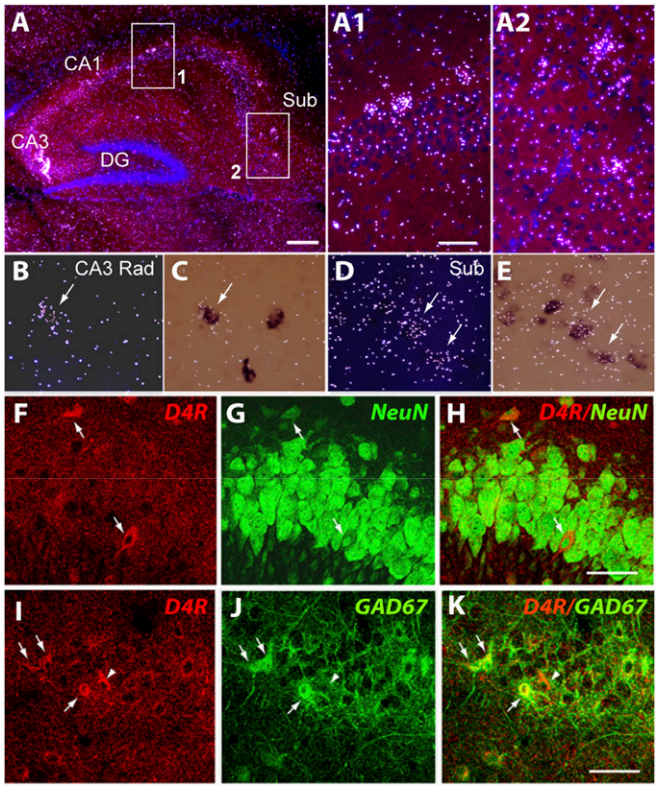

Fig. 3. D4R mRNA and protein expression in the hippocampus is highest in GABAergic interneurons. (*A*) Representative ISH low-magnification dark-field images of the hippocampus showing D4R mRNA-expressing neurons. Areas outlined by rectangles are magnified in *A1* and *A2* and show accumulation of grains over sparse cells in the CA1 and the subiculum. (B–E) DISH analysis using ^{35}S-labeled D4R and digoxigenin-labeled GAD67 probes in the CA3 (*B* and *C*) and the subiculum (*D* and *E*), revealing colocalization of both transcripts in a subset of neurons (arrows). (*F–K*) Double IFH in adult hippocampal sections for D4R and either the neuronal marker NeuN or GAD67. (*F–H*) Colocalization with NeuN shows that D4R immunofluorescence is confined to neurons located mostly in the *strata pyramidale* and *oriens*, the former consistent with basket cells. (*I–K*) In CA3, D4R-immunolabeling is observed in GAD67-positive interneurons (arrows); however, occasionally expression is observed in cells that are negative or labeled lightly for GAD67. (Scale bars, 100 μm.)

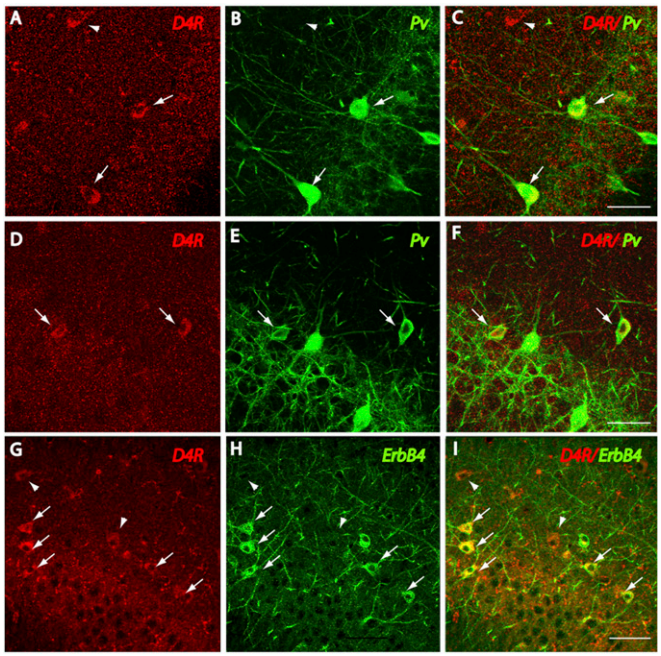

Fig. 4. D4 receptors are expressed in parvalbumin- and ErbB4-positive interneurons. Sample images of double IFH for D4R, and PV or ErbB4. D4R immunolabeling is observed in PV+ neurons in both CA1 (*A–C*) and CA3 (*D–F*) areas of the hippocampus (arrows). The localization of these PV+ neurons within or near the pyramidal layer suggests they represent basket cells. (*G–I*) A large proportion of D4R-positive cells in CA3 express ErbB4 (arrows). Note that there are D4R-immunopositive cells in both CA1 and CA3 areas that do not express PV or ErbB4 (arrowheads); refer to *Results* for quantification in CA1–CA3 and dentate. (Scale bars, 100 μm.)

oscillations (19), and the importance of both receptors for reversal of long-term potentiation (18), we reasoned that D4R activation might be necessary to mediate the effects of NRG-1 on KA-induced γ oscillations. To test this hypothesis, we tested NRG-1β (2 nM) in the presence of L-745,870. When applied alone, NRG-1β increased the power of γ oscillations to 266% (KA: 3.50E-08 ± 0.43 E-08 V^2 vs. KA+ NRG-1β: 8.63E-08 ± 1.28E-08 V^2; $n = 8$, $P = 0.007$; Fig. 5 *A* and *D*), an increase that is significantly bigger than the one produced by D4R activation (Fig. 2 *B* and *E*). In contrast, in the presence of L-745,870 (50 nM), NRG-1β augmented γ oscillation power to only 141% (KA+L-745,870: 1.78E-08 ± 5.16E-09 V^2 vs. KA+L-745,870+NRG-1: 2.51E-08 ± 7.84E-09 V^2; $n = 8$, $P = 0.046$; Fig. 5 *B* and *D*). The attenuation of the NRG-1 effect on γ oscillation power by L-745,870 was significant ($P = 0.0022$; Fig. 5*D*). As indicated earlier, L-745,870 had no significant effect on γ oscillation power on its own, indicating that NRG-1β effects on γ oscillation power are dependent on D4R activation.

We also analyzed the effects of the efficacious atypical antipsychotic clozapine because it preferentially targets DA receptors, particularly D4Rs (it additionally affects muscarinic and 5HT receptors). As shown in Fig. 5*C*, clozapine (2 μM) strongly attenuated the NRG-1β effects on γ oscillation power from 266% to 134% (KA+clozapine: 9.41E-09 ± 3.31 E-09 V^2 vs. KA+clozapine+NRG-1: 7.46E-09 ± 2.82E-09 V^2; $n = 5$, $P = 0.04$; Fig. 5*D*), therefore mimicking the effects observed with the highly selective D4R antagonist L-745,870.

Triple Immunofluorescence Identifies PV+ Interneurons That Coexpress D4 and ErbB4 Receptors. We used triple IFH for PV, D4R, and ErbB4 to test the possibility that NRG/ErbB4 and DA/D4R signaling pathways may converge on the same neurons. As shown in Fig. 5 *E–H*, we found that D4R- and ErbB4-immunoreactivity indeed overlap in the soma and proximal dendrites of a subset of PV+ interneurons in the *stratum pyramidale* of CA3 that are likely fast-spiking GABAergic basket or bistratified cells, on the basis of their location and our previous results (28). These results are consistent with the idea that the NRG/ErbB4 and DA/D4R signaling pathways may converge in a subset of interneurons that regulate γ frequency oscillations and therefore have important implications for the cellular and network activities altered in schizophrenia and other psychiatric disorders.

Discussion

Our main findings are that D4R activation increases KA-induced γ oscillation power in the hippocampus, that NRG-1-induced increases in γ oscillation power are significantly reduced by a selective D4R antagonist and by clozapine, and that ErbB4 and D4 receptors are coexpressed in PV+ interneurons in *stratum pyramidale* of CA3. These data are consistent with the idea that convergence of both signaling pathways in GABAergic interneurons underlies this modulation of γ oscillations. This cross-

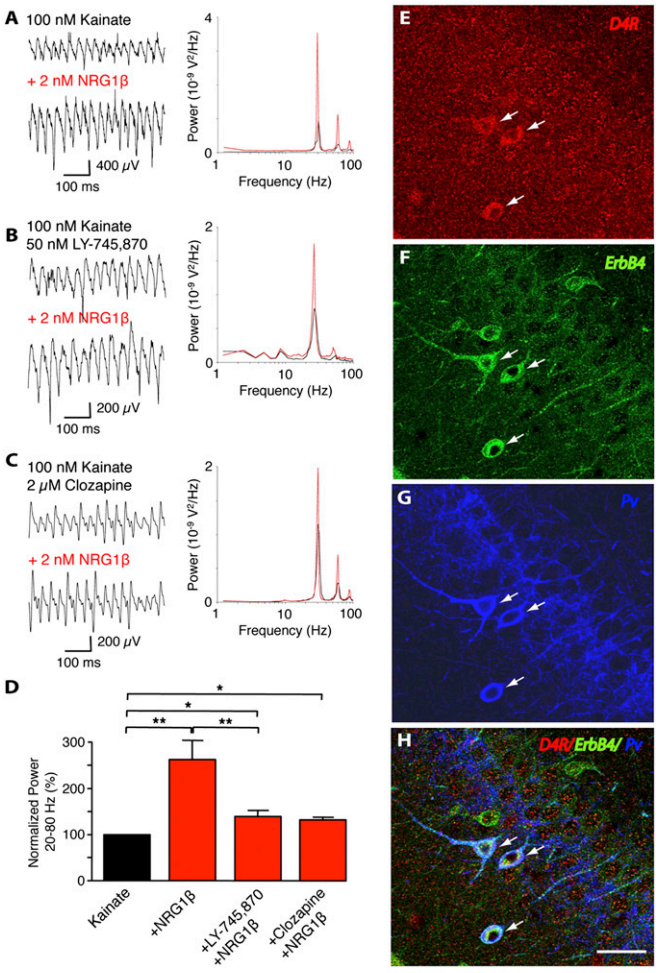

Fig. 5. Convergence of ErbB4 and D4R signaling in parvalbumin-positive interneurons. Representative sample traces (*Left*) and power spectra (*Right*) of KA-induced γ oscillations in rat slices. NRG-1β (2 nM) was tested in combination with (*A*) KA alone ($n = 8$), (*B*) KA and L-745,870 (50 nM; $n = 8$), or (*C*) KA and clozapine (2 μM; $n = 5$). (*D*) Summary analysis of normalized data from *A–C*. Values for KA treatment are set as 100%. (*E–H*) Sample images of triple IFH for D4R, ErbB4 and PV in hippocampal CA3. Overlay image showing PV+ neurons that also coexpress D4 and ErbB4 receptors (*arrows*). (Scale bar, 100 μm.)

NEUROSCIENCE

talk, taken together with the fact that polymorphisms in the NRG-1 and ErbB4 genes are associated with endophenotypes and risk for schizophrenia, that γ oscillations are altered in patients with the disorder, that reductions in PV+ interneurons are reported in postmortem brains of affected individuals, and that PV+ basket cells are thought to be critical for working memory and other cognitive functions, should stimulate renewed attention to drugs modulating D4R function to potentially ameliorate cognitive deficits in psychiatric disorders.

Differential Function of DA Receptors in the Hippocampus. By using a combination of antagonists and agonists with specificity for D1/D5, D2/D3, and D4 receptors, we revealed a selective effect of D4R activation on KA-induced γ oscillation power. In an earlier study (29), D1/D5 receptor activation produced a decrease in carbachol-induced γ oscillations. We, in contrast, did not find that D1/D5 receptor activation decreases KA-induced γ oscillation power. On the basis of our observations (Figs. 1 and 2), we suggest instead that D1/D5 receptor activity can counteract the D4R-mediated increase in γ oscillation power, consistent with studies showing bidirectional effects of DA (22, 23). Future studies will be necessary to determine whether the opposing actions of D1/D5 and D4 receptor activity in the hippocampus result from their differential cellular expression in glutamatergic (16) and GABAergic interneurons (Fig. 3) (24, 25), respectively, or from the opposing coupling of these receptors to different downstream signaling pathways.

Significance of ErbB4 and D4 Receptor Expression in PV+ GABAergic Interneurons for γ Oscillations. Alterations in DA, glutamate, and GABA neurotransmission, and in particular the function of fast-spiking PV+ interneurons, have been implicated in the observed deficits in neuronal network function that may account for reductions in cortical and hippocampal γ oscillation power and for functional disconnectivity in neural circuits observed in schizophrenia (2, 15, 30). A recent optogenetic study in the frontal cortex of mice supports the hypothesis that the excitatory/inhibitory balance critically regulates cortical network function and behaviors associated with psychiatric disorders (31), and that this balance can be modulated by DA (15).

Expression of ErbB4 receptors is restricted to ∼50% of GABAergic PV+ interneurons in the rodent hippocampus (20), whereas in the frontal cortex most ErbB4-positive interneurons also express PV (21). Interestingly, in ErbB4 null mice, enhancement of KA-induced γ oscillation power by NRG-1 is absent, and there is an ∼60% reduction in endogenous γ oscillation power that coincides with a loss of PV+ interneurons (19). The observation that D4Rs are expressed in a subset of ErbB4-expressing PV+ interneurons in CA1 and CA3 suggests that NRG/ErbB4 and D4R signaling pathways converge on the same fast-spiking basket cells to regulate γ oscillation power. Although overlap between D4 and ErbB4 receptors is partial—possibly accounting for the different efficacies between D4R agonists and NRG1 to increase γ oscillation power—it should be emphasized that D4R activity is critical for NRG1 to exert its full effect on γ oscillation (Fig. 5).

What are the potential mechanisms that mediate the effects of ErbB4 and D4Rs on KA-induced γ oscillations? Both signaling pathways acutely regulate NMDA and AMPA receptor trafficking (18, 32), and ErbB4Rs are closely associated with glutamatergic synapses on PV+ interneurons (17). Even though NMDA and AMPA receptors are not necessary for some types of γ oscillations (8), γ power can be increased through regulation of AMPA and/or NMDA receptors in PV+ interneurons by modulating excitatory/inhibitory balance and optimizing phase-synchronization of action potential discharges (31). Likewise, regulation of voltage-gated ion channels such as Kv7 can produce changes in γ oscillation power by regulating neuronal γ phase-synchrony (8, 9). $GABA_A$ receptors are another potential downstream target of D4Rs, which are expressed in both glutamatergic and inhibitory neurons in the PFC (24, 25). Perfusion of acute PFC slices and cultured neurons with PD168077 (30 μM) was reported to decrease surface expression of $GABA_A$ receptors (33). Because $GABA_A$ receptor modulation affects γ oscillation frequency (8), and we did not observe any such changes with PD168077, we lack evidence for $GABA_A$ receptor involvement. Although our results clearly show that NRG's effects on γ oscillation power require the activity of ErbB4 and D4 receptors, additional studies will be necessary to understand the underlying ionic currents regulated by both receptors in PV+ interneurons.

Psychiatric Disorders, γ Oscillations, and the Potential Use of D4R-Targeting Drugs to Modulate Cognitive Functions. Our studies showing that increases in γ oscillation power in response to NRG1 require the activity of ErbB4 and D4 receptors suggests an important nexus where genes and network activity associated with psychiatric disorders intersect in PV+ neurons. Genetic variants of *NRG1* and *ERBB4* constitute risk factors for schizophrenia (17) and associated endophenotypes (34), and the *DRD4*-7R functional variant is a risk factor for ADHD (35) and is associated with deficits in attention, working memory, and γ band activity (36). These results suggest that alterations in D4R function may underlie endophenotypes shared by both disorders. Importantly, mice harboring targeted mutations of NRG-1 and ErbB4 exhibit behavioral deficits (including working memory) similar to other rodent models for schizophrenia (37, 38), and NRG-1 hypomorphs respond positively to clozapine administration (37), suggesting that D4R modulation underlies these behaviors. Pharmacological and genetic studies targeting D4Rs in primates and rodents also support a critical role of the receptor in cognitive processes (36, 39, 40). Therefore, our prior (18) and present studies on the importance of the NRG/ErbB and D4R signaling for synaptic plasticity and γ oscillations, respectively, suggest that functional interactions between both signaling pathways may be critically important for cognitive functions.

Although first ("typical") and second ("atypical") generation antipsychotics have generally demonstrated efficacy to treat positive symptoms of schizophrenia, in most part, they have failed to improve symptoms that involve affect and social function, as well as working memory and other cognitive functions (41). The important association of γ oscillations with attention, working memory, and other cognitive functions (1), and their perturbation in numerous psychiatric disorders (2, 3, 42, 43), underscores the potential therapeutic value of targeting networks that generate and regulate γ oscillations. We propose, on the basis of the functional properties and expression of ErbB4 and D4Rs in PV+ interneurons in the dorsal lateral prefrontal cortex (21, 25) and hippocampus (this study), as well as their effects on KA-induced γ oscillations and plasticity (18, 19), that these receptors are well situated to modulate network activity that impacts cognition. The fact that D4R-specific antagonists were found to be ineffective as *antipsychotics* for the treatment of schizophrenia (44, 45) does not negate the potential use of D4R targeting drugs to treat cognitive deficits, as suggested by studies in primates and rodents (39, 40, 46, 47). The colocalization of D4 and ErbB4 receptors on PV+ interneurons, activity of which is critically important for regulating excitatory/inhibitory balance and cognitive functions (31), perhaps by optimizing "signal-to-noise" ratio of cortical microcircuits (15), makes these receptor systems attractive targets for modulating network activities that underlie cognition.

Materials and Methods

Animals. Procedures for the care and use of laboratory animals were approved and according to the Stockholms Norra Djurförsöksetiska Nämnd, Sweden, and the National Institutes of Health and Texas A&M University.

Electrophysiology. Local field potential recordings from horizontal Sprague-Dawley rat hippocampal slices (300 μm) in CA3 were performed at 32 °C in a submerged recording chamber (*SI Materials and Methods*), essentially as described previously (19). Drugs were obtained from Sigma-Aldrich (kainic acid), R&D Systems (NRG-1β), and Tocris (all other drugs). Fast Fourier transforms for power spectra were computed from 60-s traces and power values obtained by integrating power spectra between 20 and 80 Hz using KaleidaGraph (Synergy Software). Statistical calculations were carried out using GraphPad Prism. Data are reported as mean ± SEM, as well as in normalized form ± SEM where normalization for the KA-only value was set to 100%.

DISH and IFH. DISH experiments were performed as previously reported (48) using C57Bl6/J mouse brain sagittal cryostat sections (20 μm) hybridized with S^{35}-CTP- and digoxigenin-labeled D4R and GAD67 cRNA probes, respectively. After alkaline phosphatase treatment for Dig, slides were exposed for 4 wk for autoradiography. Double and triple immunofluorescence histochemistry experiments were essentially as previously described (20) using an affinity-purified N-terminal rabbit antibody (24); specificity was determined by immunofluorescence analysis of cultured hippocampal neurons transfected with D1-D5 receptor cDNA expression vectors (*SI Materials and Methods*). Multiple IFH was performed in 3-wk-old rat vibratome coronal sections (50 μm) using antibodies for D4R (2 μg/mL), GAD67 (1:5,000; mouse mAb 5406; Millipore), PV (1:6,000; mAb P19; Sigma), and ErbB4 (3 μg/mL; mAb-77; ThermoScientific). Confocal immunofluorescence images were obtained with 20× and 63× oil objectives.

ACKNOWLEDGMENTS. We thank V. Schram (National Institute of Child Health and Human Development) and C. Smith (National Institute of Neurological Disorders and Stroke) for assistance at the microscopy cores, and Joerg Neddens for constructive comments. This work was supported by a Karolinska Institute doctoral fellowship (R.H.A.), the Karolinska Institute/National Institutes of Health Graduate Partnerships Program (A.J.), and grants from the Swedish Research Council (Vetenskapsrådet), the Swedish Brain Fund (Hjärnfonden), the Strategic Program in Neurosciences at Karolinska Institute (StratNeuro), the Swedish Medical Association (Svenska Läkaresällskapet), and the Karolinska Institute (A.F.), and the NICHD Intramural Research Program (P.A.H., I.K., D.V., and A.B.).

1. Uhlhaas PJ, Singer W (2010) Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* 11:100–113.
2. Lewis DA, Fish KN, Arion D, Gonzalez-Burgos G (2011) Perisomatic inhibition and cortical circuit dysfunction in schizophrenia. *Curr Opin Neurobiol* 21:866–872.
3. Minzenberg MJ, et al. (2010) Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. *Neuropsychopharmacology* 35:2590–2599.
4. Colgin LL, Moser EI (2010) Gamma oscillations in the hippocampus. *Physiology (Bethesda)* 25:319–329.
5. Whittington MA, Traub RD, Jefferys JG (1995) Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation. *Nature* 373:612–615.
6. Fisahn A, Pike FG, Buhl EH, Paulsen O (1998) Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature* 394:186–189.
7. Hájos N, et al. (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci* 12:3239–3249.
8. Fisahn A, et al. (2004) Distinct roles for the kainate receptor subunits GluR5 and GluR6 in kainate-induced hippocampal gamma oscillations. *J Neurosci* 24:9658–9668.
9. Leão RN, Tan HM, Fisahn A (2009) Kv7/KCNQ channels control action potential phasing of pyramidal neurons during hippocampal gamma oscillations in vitro. *J Neurosci* 29:13353–13364.
10. Bartos M, Vida I, Jonas P (2007) Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci* 8:45–56.
11. Fuchs EC, et al. (2007) Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. *Neuron* 53:591–604.
12. Korotkova T, Fuchs EC, Ponomarenko A, von Engelhardt J, Monyer H (2010) NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* 68:557–569.
13. Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009) Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 459:698–702.
14. Carlen M, et al. (2011) A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol Psychiatry* 17:537–548.
15. Winterer G, Weinberger DR (2004) Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci* 27:683–690.
16. Jay TM (2003) Dopamine: A potential substrate for synaptic plasticity and memory mechanisms. *Prog Neurobiol* 69:375–390.
17. Buonanno A (2010) The neuregulin signaling pathway and schizophrenia: From genes to synapses and neural circuits. *Brain Res Bull* 83:122–131.
18. Kwon OB, et al. (2008) Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. *Proc Natl Acad Sci USA* 105:15587–15592.
19. Fisahn A, Neddens J, Yan L, Buonanno A (2009) Neuregulin-1 modulates hippocampal gamma oscillations: Implications for schizophrenia. *Cereb Cortex* 19:612–618.
20. Neddens J, Buonanno A (2010) Selective populations of hippocampal interneurons express ErbB4 and their number and distribution is altered in ErbB4 knockout mice. *Hippocampus* 20:724–744.
21. Neddens J, et al. (2011) Conserved interneuron-specific ErbB4 expression in frontal cortex of rodents, monkeys, and humans: Implications for schizophrenia. *Biol Psychiatry* 70:636–645.
22. Trantham-Davidson H, Neely LC, Lavin A, Seamans JK (2004) Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. *J Neurosci* 24:10652–10659.
23. Goto Y, Otani S, Grace AA (2007) The Yin and Yang of dopamine release: A new perspective. *Neuropharmacology* 53:583–587.
24. Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR (1997) Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res* 752:26–34.
25. Mrzljak L, et al. (1996) Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature* 381:245–248.
26. Defagot MC, Malchiodi EL, Villar MJ, Antonelli MC (1997) Distribution of D4 dopamine receptor in rat brain with sequence-specific antibodies. *Brain Res Mol Brain Res* 45:1–12.
27. Bunnett NW, Cottrell GS (2010) Trafficking and signaling of G protein-coupled receptors in the nervous system: implications for disease and therapy. *CNS Neurol Disord Drug Targets* 9:539–556.
28. Andersson R, Johnston A, Fisahn A (2012) D4 receptor-dependant augmentation of hippocampal gamma oscillations by increased spiking coherence in fast-spiking interneurons. *PLoS ONE* 7(7):e40906.
29. Weiss T, Veh RW, Heinemann U (2003) Dopamine depresses cholinergic oscillatory network activity in rat hippocampus. *Eur J Neurosci* 18:2573–2580.
30. Lisman JE, et al. (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* 31:234–242.
31. Yizhar O, et al. (2011) Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* 477:171–178.
32. Yuen EY, Yan Z (2009) Dopamine D4 receptors regulate AMPA receptor trafficking and glutamatergic transmission in GABAergic interneurons of prefrontal cortex. *J Neurosci* 29:550–562.
33. Graziane NM, Yuen EY, Yan Z (2009) Dopamine D4 receptors regulate GABAA receptor trafficking via an actin/cofilin/myosin-dependent mechanism. *J Biol Chem* 284:8329–8336.
34. Greenwood TA, et al. (2011) Analysis of 94 candidate genes and 12 endophenotypes for schizophrenia from the Consortium on the Genetics of Schizophrenia. *Am J Psychiatry* 168:930–946.
35. DiMaio S, Grizenko N, Joober R (2003) Dopamine genes and attention-deficit hyperactivity disorder: A review. *J Psychiatry Neurosci* 28:27–38.
36. Demiralp T, et al. (2007) DRD4 and DAT1 polymorphisms modulate human gamma band responses. *Cereb Cortex* 17:1007–1019.
37. Stefansson H, et al. (2002) Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 71:877–892.
38. Shamir A, et al. (2012) The importance of the NRG-1/ErbB4 pathway for synaptic plasticity and behaviors associated with psychiatric disorders. *J Neurosci* 32:2988–2997.
39. Arnsten AF, Murphy B, Merchant K (2000) The selective dopamine D4 receptor antagonist, PNU-101387G, prevents stress-induced cognitive deficits in monkeys. *Neuropsychopharmacology* 23:405–410.
40. Young JW, Powell SB, Scott CN, Zhou X, Geyer MA (2011) The effect of reduced dopamine D4 receptor expression in the 5-choice continuous performance task: Separating response inhibition from premature responding. *Behav Brain Res* 222:183–192.
41. Insel TR (2010) Rethinking schizophrenia. *Nature* 468:187–193.
42. Spencer KM (2008) Visual gamma oscillations in schizophrenia: Implications for understanding neural circuitry abnormalities. *Clin EEG Neurosci* 39:65–68.
43. Herrmann CS, Demiralp T (2005) Human EEG gamma oscillations in neuropsychiatric disorders. *Clin Neurophysiol* 116:2719–2733.
44. Bristow LJ, et al. (1997) Schizophrenia and L-745,870, a novel dopamine D4 receptor antagonist. *Trends Pharmacol Sci* 18:186–188.
45. Corrigan MH, Gallen CC, Bonura ML, Merchant KM; Sonepiprazole Study Group (2004) Effectiveness of the selective D4 antagonist sonepiprazole in schizophrenia: A placebo-controlled trial. *Biol Psychiatry* 55:445–451.
46. Zhang K, et al. (2004) Regulation of working memory by dopamine D4 receptor in rats. *Neuropsychopharmacology* 29:1648–1655.
47. Braszko JJ (2009) Dopamine D4 receptor antagonist L745,870 abolishes cognitive effects of intracerebroventricular angiotensin IV and des-Phe(6)-Ang IV in rats. *Eur Neuropsychopharmacol* 19:85–91.
48. Son JH, Winzer-Serhan UH (2009) Signal intensities of radiolabeled cRNA probes used alone or in combination with non-isotopic in situ hybridization histochemistry. *J Neurosci Methods* 179:159–165.